Germination of *Styrax japonicus* Seeds as Influenced by Storage and Sowing Conditions

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Abstract

This study evaluated the effect of storage and sowing conditions on seed germination of *Styrax japonicus* Sieb. et. Zucc, an ornamental tree with seeds that exhibit double dormancy. The germination of freshly harvested seeds was compared with seeds that had been stored dry at 20 °C for a year before sowing. After sowing, potted seeds were placed in an air-refrigerated greenhouse maintained 18.5/18 °C. Pots were kept for 0, 1, 2, 3, or 4 months in the greenhouse for warm stratification (WS), and then moved to a 5.5 °C cooler for 3 months for cold stratification (CS). After CS, pots were moved back to the greenhouse. None of the fresh or dried seeds that received CS immediately after sowing germinated. Germination of fresh and dried seeds did not differ when the seeds received WS for at least 1 month. Dry seeds, that received 3 or 4 months of WS, had a lower germination percentage than fresh seeds. The effect of moisture after sowing on the germination of freshly harvested seeds was determined by either watering or not watering the seeds before they were placed in the cooler. In addition seeds under the moist treatment were watered as needed to keep the medium moist. More than 60% of the seeds that received CS for at least for 1 month germinated. A radicle did not emerge through the seed coat after warm stratification, or at the completion of cold stratification. It is recommended that *Styrax* seeds receive 1 month of WS, followed by 2 months of CS.

INTRODUCTION

Styrax japonicus is a small, low branched tree that produces white flowers with yellow stamens (Dirr, 1990) (Fig. 1). This species can be propagated from stem cuttings or seeds. Seeds that are sown in the fall without a sequential warm and cold stratification may germinate the second spring (Dirr, 1990; Kwon, 1995). Kwon (1995) suggested that a radicle might emerge after 3 months of WS, and then enter a dormancy. Stratification of seeds using warm and cold temperatures is required to break seed dormancy for many woody plants to improve seed germination (Young and Young, 1992). Seed dormancy is mediated, in part, by a hard seed coat or testa, or by a dormant embryonic axis and cotyledons (Bandyopadhyay et al., 1999). To improve germination *Styrax* seeds should be stored in a moist, warm environment for 3 (Kwon, 1995) or 5 months (Dirr, 1990), and then stored at low temperatures for 3 or 4 months (Dirr, 1990; Kwon, 1995).

Seeds that are sown immediately after collection may germinate by the following spring, suggesting that non-fresh seeds may take longer to germinate (Dirr, 1990). Thus, this study aimed to determine (a) the effect of dry storage duration at 20 °C, and (b) the effect of moisture during storage at 18.5/18 °C on germination of *Styrax* seeds.

MATERIALS AND METHODS

Dry Storage Duration

Seeds (NA 60191) from the U. S. National Arboretum, Woody Landscape Plants Germplasm Repository at Glenn Dale, MD, were harvested on Oct. 3, 2000. A group from these seeds was sown on Nov. 8 and held for 0, 1, 2, 3, or 4 months without watering (dry) in an air-refrigerated greenhouse maintained at 18.5/18 °C. After dry storage, pots were watered and kept moist in the greenhouse for 2 months of warm stratification (WS) and moved to a 5 °C cooler for cold stratification (CS). Another group of seeds was sown, watered, kept moist for 0, 1, 2, 3, or 4 months of WS and then moved

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to a cooler for 3 months of CS. Forty seeds were sown per 15 cm pots filled with ProMix BM (Stamford, Conn). Three pots were used per treatment. After CS, pots were moved to the greenhouse where the number of seeds that germinated was recorded.

Peak seed germination was defined as the maximum number of seeds that germinated during the test period. This number was calculated as a percentage, i.e., (no. of germinated seeds/ total number of seeds per pot)*100. Weeks for germination peak was defined as the number of weeks that elapsed from the start of the experiment, i.e., when put into the greenhouse, until reaching peak seed germination.

These two dependent variables were analyzed using a 2-way analysis of variance (ANOVA), with interaction. Duration of storage (in months) and storage condition (moist vs. dry) were tested as main effects. The control group, i.e., those seeds that were sown without any WS were not included in the analysis to keep a balanced design. Means of the dependent variables and the standard error of the means (SEM) were obtained using the LSMEANS procedure within SAS (SAS Institute 1999). Means were compared using contrasts at $P < 0.05$. Because germination was recorded over time, germination was modeled as a polynomial function of time using a mixed model, repeated measures analysis with an autoregressive order one covariance structure. Parameter estimates were obtained using the Solution option in the Model statement.

Moisture During Storage

Seeds (NA 60191) were harvested on Sept. 24, 1999 and Oct. 3, 2000, and seeds were dry stored at 20 °C until sowing Nov. 8, 2000 until sowing. After sowing, pots were watered and exposed to 0, 1, 2, 3, or 4 months of WS, followed by CS at 5 °C for 3 months. Forty seeds were sown per 15 cm pots, filled with ProMix BM (Stamford, Conn). Three pots were used per treatment. Pots were moved to a greenhouse where the number of seeds that germinated were recorded weekly. Data were analyzed using a 2-way ANOVA, with interaction. The year when seeds were collected and months of WS were treated as main effects. Because germination was recorded over time, germination was modeled as a polynomial function of time using a 2-way, repeated measures analysis of variance with an autoregressive order one covariance structure. Parameter estimates were obtained using the Solution option in the Model statement.

RESULTS

Dry Storage Duration

Control seeds that were kept in a 18.5/18 °C greenhouse did not germinate by the time the experiment was completed on Sept. 10 (Table 1). Examination of non-germinated seeds revealed that the seed coat was intact in most of the seeds, yet a radicle was visible through the seed coat in less than 10% of the non-emerged seeds (Fig. 2). The mean peak germination was significantly influenced by the interaction between storage duration and moisture ($F = 55.13$; df = 3,16; $P = <0.0001$; $N = 24$). After 1 month of moist WS, more than 70% of the seeds had germinated. More than 53.3% of seeds germinated when receiving moist WS. Germination of seeds receiving dry WS decreased to 28.3% as the storage period increased to 4 months (Fig. 3).

The mean number of weeks to reach the germination peak was not significantly influenced by the interaction between storage duration and moisture ($F = 2.91$; df = 3,16; $P = 0.0666$; $N = 24$). The number of weeks until germination peak was significantly influenced by storage moisture as a main effect ($F = 5.40$; df = 1,16; $P = 0.0336$; $N = 24$). Regardless of storage duration, seeds that received dry WS (6.33 ± 0.22 week) took longer to reach their peak germination than seeds that received moist WS (5.58 ± 0.22 week). Germination regressed over time was significantly influenced by the interaction between storage duration and storage moisture ($F = 124.24$; df = 8, 65.9; $P < 0.0001$; $N = 168$). The quadratic effect was significant, thus included in the model ($F = 16.17$; df = 8, 111; $P < 0.0001$; $N = 168$). Regression slopes were significantly different among treatment combinations.

Moisture During Storage

The germination peak of seeds harvested in 1999 was higher than for those harvested in 2000, except for seeds that were stored for 1 month were similar (Table 2). Seed germination was significantly influenced by the interaction of seed harvest year and duration of seed storage ($F = 21.54$; df = 4,20; $P = <0.0001$; $N = 30$). Germination was significantly decreased when seeds harvested in 2000 were stored for 3 or 4 months. The mean number of weeks to reach the germination peak was not significantly influenced by the interaction between year and months ($F = 1.75$; df = 4,20; $P = 0.1787$; $N = 30$). Yet, both year ($F = 8.00$; df = 1,20; $P = 0.0104$), and months ($F = 104.50$; df = 4,20; $P < 0.0001$) were significant as main effects. The mean number of weeks to reach the germination peak was significantly longer for those seeds collected in 2000 (4.07 ± 0.13), than for seeds collected in 1999 (3.53 ± 0.13), and for seeds stored for 3 months.

Germination regressed over time was significantly influenced by the interactive effect of year and months ($F = 3.47$; df = 4, 134; $P = 0.0098$; $N = 180$). The quadratic effect was significant, and thus included in the model ($F = 29.81$; df = 10, 134; $P < 0.0001$; $N = 180$). Regression slopes were significantly different among treatment combinations.

DISCUSSION AND CONCLUSIONS

The maximum percent germination decreased when seeds were dry stored during WS treatment. Roh (unpublished data) noted that seeds harvested in September, 1998 and stored dry at 20 °C for 6 months showed greater than 90% germination when seeds received 1 month WS followed by 2 or 3 months of CS. It is not apparent why the percent germination of seeds harvested in Oct. 2000 dropped to 28.3% when stored dry for 4 months, while the highest germination peak was about 70%. The low percent germination of seeds harvested in 2000 does not appear to be due to the seed maturity, since seeds harvested 14 weeks after flowering were considered mature (Roh, unpublished data). Seeds were harvested more than 14 weeks after flowering. Although differences appear to be due to yearly variances, it is not clear whether seed germination could be improved if a second cycle of WS and CS were given to the seeds.

Styrax seeds need warm moist stratification for at least one month followed by cold stratification to improve germination. Without any WS, seeds would not germinate. For example, seeds that were sown in the fall germinated in the second spring in the field. This could possibly be related to the moisture level of the seeds during the fall before seeds were exposed to cold. If seeds were on the ground dry, WS requirements could not be fulfilled, and thus seeds may not be able to respond to CS treatment. The low germination of seeds that were harvested in 2000 and stored dried for 4 months could be due to an unknown physiological process in the seeds, because seeds that were harvested in 1999 showed higher than 80.8% germination (Fig. 4) while seeds harvested in 2000 showed less than 53.5%.

The results from this study suggest that seeds should be sown in the same year that seeds are harvested to avoid any possible reduction in the germination percentage. At least one month of warm stratification is required, followed by 3 months of cold stratification (5 °C) to improve germination. Further studies are needed to investigate the loss of seed viability observed in seeds that were harvested in 2000.

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Tables

Table 1. Effects of storage duration and moisture on seed germination peak and on the number of weeks to reach germination peak.

Month in storage	Moisture during storage	Germination peak (%)[z]	Wk to peak germination[z]	Regression equation
0	Dry	0		
	Moist	0		
1	Dry	70.8 ± 2.2 a	6.3 ± 0.45	$Y = -36.08 + 31.18X - 2.24X^2$
	Moist	53.3 ± 2.2 c	6.3 ± 0.45	$Y = -27.24 + 25.41X - 2X^2$
2	Dry	59.2 ± 2.2 bc	7.0 ± 0.45	$Y = -24.78 + 20.87X - 1.24X^2$
	Moist	65.0 ± 2.2 ab	5.0 ± 0.45	$Y = -37.57 + 36.39X - 3.13X^2$
3	Dry	41.7 ± 2.2 d	6.7 ± 0.45	$Y = -14.98 + 14.84X - 0.98X^2$
	Moist	61.7 ± 2.2 b	5.3 ± 0.45	$Y = -33.69 + 31.52X - 2.55X^2$
4	Dry	28.3 ± 2.2 e	5.3 ± 0.45	$Y = -11.86 + 9.81X - 0.55X^2$
	Moist	66.7 ± 2.2 a	5.7 ± 0.45	$Y = -31.49 + 30.77X - 2.39X^2$

[y] Mean ± standard error of the means. Means within each column, followed by the same letters are not significantly different at $P<0.05$.

Table 2. Effect of seed harvest year and months in storage on seed germination peak.

Seed harvest year	Storage duration at 18.5/18°C	Germination peak (%) [z]	Regression equation
1999	0	0	
	1	70.8 ± 2.4 b	$Y = -43.84 + 48.05x - 4.87x^2$
	2	85.0 ± 2.4 a	$Y = -50.78 + 52.41x - 4.96x^2$
	3	80.8 ± 2.4 a	$Y = -49.42 + 58.24x - 6.19x^2$
	4	85.0 ± 2.4 a	$Y = -44.82 + 55.35x - 5.70x^2$
2000	0	0	
	1	72.5 ± 2.4 b	$Y = -47.33 + 45.43X - 4.22X^2$
	2	75.8 ± 2.4 b	$Y = -24.00 + 19.66X - 0.47X^2$
	3	52.5 ± 2.4 c	$Y = -32.48 + 32.46X - 3.05X^2$
	4	53.3 ± 2.4 c	$Y = -27.06 + 28.79X - 2.55X^2$

[z] Mean ± standard error of the means. Means within each column, followed by the same letters are not significantly different from each other.

Figures

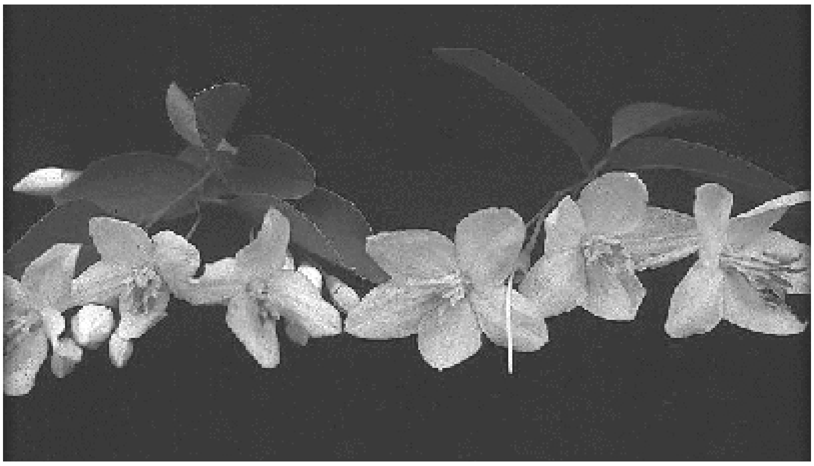

Fig. 1. Flowers of *Styrax japonicus* .

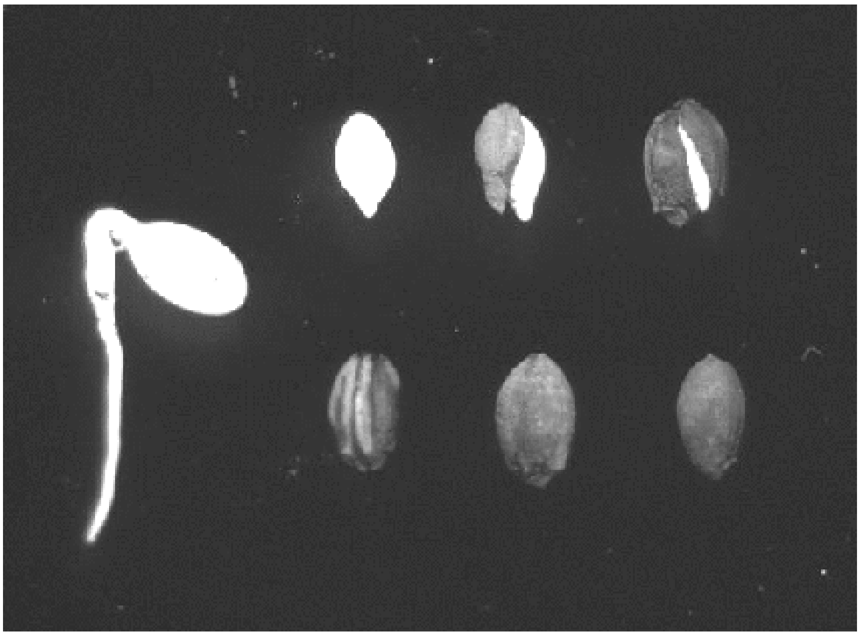

Fig. 2. *Styrax* seeds at the end of experiment. A few of seeds had a radicle emerged.

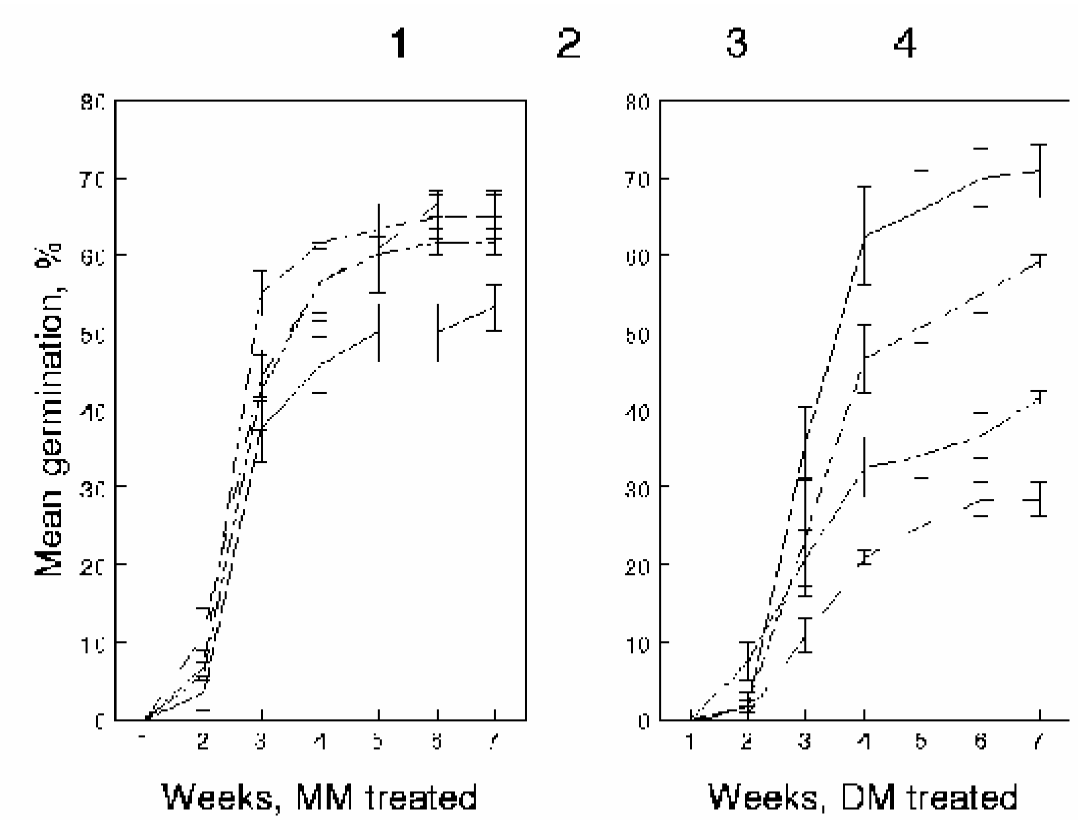

Fig. 3. *Styrax japonicus* seed germination as influenced by storage period either under moist condition (MM) or dry condition (DM) for 1, 2, 3, or 4 months.

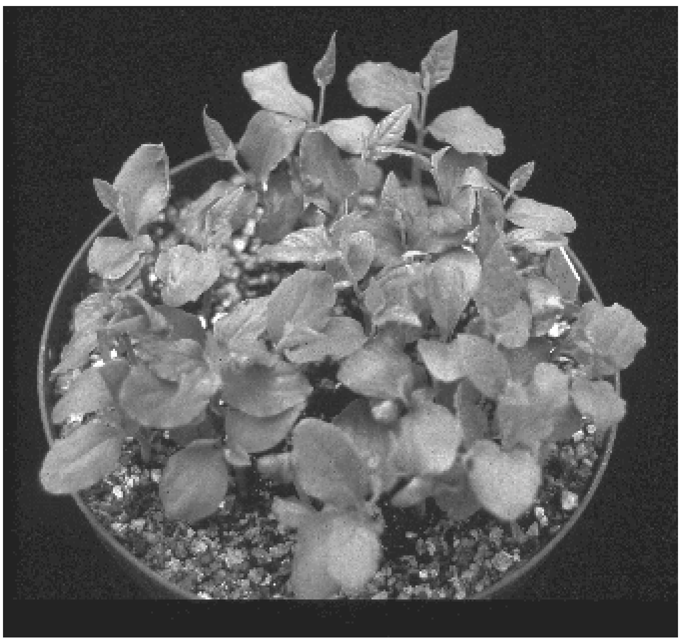

Fig. 4. Uniform and well germinated *Styrax* seedlings.